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13. ABSTRACT *(Maximum 200 words)*

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Results. None of the eight soldiers had classic signs or symptoms of visceral leishmaniasis (kala-azar). Seven soldiers had unexplained fever, chronic fatigue, malaise, cough, intermittent diarrhea, or abdominal pain that began up to seven months after they returned to the United States; one had no symptoms. Five had adenopathy or mild, transient hepatosplenomegaly. None had cutaneous manifestations. Diagnoses were made by bone marrow aspiration (seven patients) or lymph-node biopsy (one patient). Six isolates have been identified as *L. tropica*, which usually causes only cutaneous disease. Of the six patients treated with sodium stibogluconate, five improved and one remained symptomatic.

Conclusions. *L. tropica* can produce visceral infection that can cause unexplained systemic illness in persons returning from areas where this organism is endemic. (N Engl J Med 1993;328:1383-7.)

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VISCERAL INFECTION CAUSED BY *LEISHMANIA TROPICA* IN VETERANS OF OPERATION DESERT STORM

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Abstract *Background.* Visceral leishmaniasis, usually caused by *Leishmania donovani,* has rarely been reported from eastern Saudi Arabia, so it was not expected to affect the soldiers of Operation Desert Storm.

Methods. We evaluated eight soldiers with visceral leishmanial infection, examining their serum with an immunofluorescent-antibody assay, examining their marrow or biopsy tissue for amastigotes with an indirect immunofluorescent-monoclonal-antibody assay, and culturing the parasites. Cultured promastigotes were isolated and characterized by isoenzyme analysis.

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THE deployment of over 500,000 soldiers to the Arabian peninsula during Operation Desert Storm exposed immunologically naive hosts to infectious pathogens uncommon in North America.[1,2] Kala-azar (visceral leishmaniasis) was thought to be rare in eastern Saudi Arabia and uncommon elsewhere in the country.[3,4]

Kala-azar, typically caused by *Leishmania donovani,* presents as a chronic febrile illness with emaciation, marked hepatosplenomegaly, pancytopenia, and hyperglobulinemia. We describe eight American soldiers who had a systemic leishmanial infection that differed from kala-azar in that the infecting organism was *L. tropica* rather than *L. donovani.* These patients did not have the classic signs or symptoms of kala-azar.

METHODS

Characteristics of the Patients

Patient 1 was evacuated from Saudi Arabia with an unknown febrile illness. Patients 2, 3, and 7 were directly referred early in the course of their illnesses because of their symptoms. Patient 4 was in the same unit as Patient 2, and his illness was diagnosed during a serologic survey. Patients 5 and 6 were in the same unit as Patient 1 and were identified during a serologic survey. Patient 8 was referred because of a febrile illness.

Laboratory Studies

Titers of antibody to leishmania were determined by immunofluorescence assays.[5-7] Mononuclear cells obtained by density sedimentation of bone marrow aspirates were analyzed by an indirect immunofluorescence assay incorporating a monoclonal antibody specific for leishmanial organisms.[8] Bone marrow samples were obtained from the iliac crest by needle aspiration. The samples were directly inoculated at the patient's bedside into a diphasic blood-agar medium with an overlay of 0.1 ml of complete Schneider's drosophila medium (GIBCO, Grand Island, N.Y.), after which they were permanently sealed.[9,10] Cultures were incubated at 25°C and evaluated microscopically (×400) daily for 40 days. The isoenzyme profiles were determined by cellulose acetate electrophoresis.[11,12] The simplified nomenclature for the genus leishmania suggested by Lainson and Shaw was used.[13]

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ILLUSTRATIVE CASE REPORTS

Visceral leishmaniasis may have an acute presentation or may appear weeks to months after exposure, as demonstrated by the following case reports. The patient numbers reflect the chronologic order in which the patients presented, and correspond to the patient numbers in Table 1.

Patient 2

Patient 2 was a 39-year-old man who reported the abrupt onset of fever, rigors, nonproductive cough, and malaise one month after his return from Saudi Arabia. He had lived in an apartment complex in Dhahran and had never traveled more than 15 miles (24 km) from the city. He presented with a temperature of 38.9°C and a pulse of 88 and did not appear to be extremely ill. Physical examination revealed diffuse abdominal tenderness without organomegaly. Laboratory data about this patient are shown in Table 2.

Patient 3

Patient 3 was a 20-year-old man who reported the onset of watery diarrhea, nausea, and diffuse abdominal pain two months after his return from Saudi Arabia. He reported no fever, weight loss, or night sweats. He had been based outside Riyadh and had worked and slept indoors most of the time. Physical examination revealed only tenderness in the right and left upper quadrants. His hematocrit was 41 percent, his white-cell count 5300 per cubic millimeter with 10 percent monocytes, and his alanine aminotransferase concentration was 82 IU per liter. Laboratory examinations for fecal leukocytes, enteric bacterial pathogens, and intestinal parasites were negative.

Over a three-week period, abdominal pain localized to the left upper quadrant, and the tip of the spleen became palpable. The patient remained afebrile, but coryza, nonproductive cough, and severe headaches associated with neck stiffness developed. His hematocrit declined to 39 percent, and his alanine aminotransferase concentration increased to 128 IU per liter.

Patient 6

Patient 6 was a 35-year-old man who reported the gradual onset of malaise, fatigue, anorexia, nausea, abdominal pain, headaches, nonproductive cough, arthralgias, and myalgias seven months after

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Table 1. Clinical Presentation of Eight Male Patients with Visceral Leishmaniasis, at the Time of Confirmatory Culture.

Patient No.	Incubation Period (Mo)	Signs and Symptoms at Presentation	Fever	Abdominal Pain*	Malaise*	Fatigue*	Physical Examination
1	2	Adenopathy	Yes	++	+	++	Hepatomegaly, splenomegaly, adenopathy
2	1–4	Fever	Yes	+	++	+	Normal findings
3	2–8	*Gastroenteritis*	No	+++	+++	+	Splenomegaly
4	2–6	None	No	No	No	No	Normal findings
5	4–12	Chronic fatigue with hepatosplenomegaly	Yes	+	+	+++	Hepatomegaly, splenomegaly
6	7–14	Chronic fatigue with adenopathy	No	+	+	+++	Hepatomegaly, adenopathy
7	1–6	Mononucleosis	Yes	+/−	+++	+	Normal findings
8	3–12	Fever of unknown origin	Yes	+	++	++	Hepatomegaly, splenomegaly

*One plus sign indicates that the patient reported the symptom when questioned by the examiner; two plus signs, that the patient himself reported the symptom without questioning; and three plus signs, that the symptom was the primary one. Patient 7, represented by the plus–minus sign, reported abdominal pain of brief duration associated with diarrhea.

returning from Saudi Arabia. Physical examination revealed tenderness of the right and left upper quadrants without organomegaly. He had served throughout Saudi Arabia and in Iraq as part of an airborne unit. Two previously identified patients (Patients 1 and 5) were from the same unit. In an epidemiologic serologic survey of his unit in August 1991, he had an antileishmanial titer of 1:32 on immunofluorescent-antibody assay.

Tests of bone marrow aspirates in November 1991 and January 1992 were negative for parasites. Disabling fatigue developed, along with nonspecific abdominal symptoms. Hepatomegaly was first noted on a physical examination in mid-February 1992, and a percutaneous liver biopsy was performed in March 1992. A histopathological evaluation showed mild chronic inflammation of the portal triads, but tissue staining and culture for parasites were negative. The hepatomegaly slowly resolved over a six-week period. A single left axillary lymph node was found to be tender in April 1992, and an excisional biopsy was performed. A touch preparation of the cut surface of the node was positive for amastigotes on indirect immunofluorescent-monoclonal-antibody assay, and the culture was positive for *L. tropica*. Thus, the diagnosis was confirmed 6 months after the onset of symptoms and 14 months after the patient's return from Saudi Arabia. At no time during this prolonged illness did he have a documented fever or an abnormal finding on standard blood tests that included complete blood counts, serum biochemical assessments, and liver-enzyme measurements.

The patient completed a 30-day course of treatment with sodium stibogluconate (20 mg per kilogram of body weight per day) without severe complications. During the four months after the end of therapy he had no improvement in his symptoms. The isolate was moderately resistant to sodium stibogluconate according to semiautomated microdilution testing.[14]

Table 2. Laboratory Data in the Study Patients.

Patient No.	Hemoglobin	Hematocrit	Aminotransferases*		Serum Antibody Titer†	
			AST	ALT	Predeployment	Postdeployment
	g/dl	%	*IU/liter*			
1	13	37	98	210	NA	1:8
2	12	35	84	122	1:8	1:16
3	13.3	39	82	128	1:8	1:32
4	16	48	46	70	1:4	1:16
5	13	39	23	37	NA	1:32
6	14	45	30	45	1:4	1:32
7	14.3	43	160	403	NA	1:64
8	15.3	44	58	139	1:4	1:32

*AST denotes aspartate aminotransferase (normal, 10 to 50 IU per liter), and ALT alanine aminotransferase (normal, 5 to 42 IU per liter).

†Titers were determined by immunofluorescent antibody assay. NA denotes serum sample not available. Predeployment samples were obtained from the bank of serum samples tested for HIV during routine armed-forces screening.

Results

The patients with visceral leishmaniasis we describe all presented between November 1990 and April 1992. Their median age was 32.5 years (range, 21 to 40), and all were men. For the maximal incubation period, defined as the interval between arrival in Saudi Arabia and the onset of symptoms, the median was 7 months (range, 2 to 14); for the minimal incubation period, defined as the time between departure from Saudi Arabia and the onset of clinical illness, the median was 2 months (range, 1 to 7). In Patient 1, the incubation period was two months, since he became ill while in Saudi Arabia.

The clinical presentations of leishmaniasis are summarized in Table 1. It had an acute presentation in Patients 1, 2, 5, and 7, with high fever (temperatures up to 40.5°C), rigors, and malaise. Other symptoms at onset were headaches, nonproductive cough, and diffuse abdominal pain. Patient 3 presented with gastroenteritis. Patient 4 had no history of illness and no symptoms at the time of bone marrow aspiration. The diagnosis was pursued because immunofluorescent-antibody assay had demonstrated a serum titer of 1:16 in a serologic survey. Patient 6 had a very nonspecific, chronic presentation. Patient 8 had low-grade fever and malaise for several months. The median duration of symptoms before diagnosis was 28 days (mean, 69; range, 5 to 180). The diagnosis was delayed because leishmaniasis had not been included in the differential diagnosis at presentation. The diagnosis was confirmed with a single bone marrow aspiration in all patients except Patient 6. The diagnosis of leishmaniasis was pursued relentlessly in this patient because of a strong epidemiologic association with confirmed cases in two others (Patients 1 and 5) and the exclusion of other diagnoses.

Patients 7 and 8 were found to have other serious diseases during extensive evaluations. Patient 7 had a sudden onset of fever, rigors, malaise, and right-lower-quadrant pain with diarrhea one month after

returning from Saudi Arabia. Twelve days after bone marrow aspiration showed amastigotes, infection with the human immunodeficiency virus (HIV) was confirmed by Western blotting. Serum obtained from this patient early in his illness at the referring hospital was negative for the virus, thus documenting seroconversion. Patient 8 had a 3-cm mass in his right kidney on computed tomography of the abdomen. He underwent nephrectomy, and renal-cell carcinoma was confirmed pathologically. He had not had flank pain, and no palpable mass or occult hematuria had been found during examinations. There was no radiographic or pathological evidence of metastatic disease.

No patient had lesions that suggested cutaneous leishmaniasis according to his history or physical examination. Physical findings were initially nonspecific in the patients with acute presentations of leishmaniasis. Even those with temperatures above 40.5°C appeared surprisingly well, remaining alert and oriented. When their temperatures declined to 38 to 39°C, patients often felt relatively well. In the patients with abdominal pain, the initially nonfocal tenderness tended to localize to the left or the right upper quadrant (or both quadrants) over several days. In Patients 1 and 6, lymphadenopathy was documented weeks after the onset of illness. Marked weight loss was documented in Patients 1 and 5.

Laboratory findings are summarized in Table 2. At the time of tissue diagnosis, seven of the eight patients had some abnormality of either their hemoglobin concentration or liver-enzyme levels. These abnormalities were minimal in some and nonspecific in all. No patient had leukopenia, thrombocytopenia, hyperglobulinemia, or an elevated erythrocyte sedimentation rate at the time of tissue diagnosis.

A serum titer above 1:16 on immunofluorescent-antibody assay, suggestive of leishmanial infection,[15] was found in the samples from five of the eight patients. We were able to obtain predeployment serum samples for five patients; the titers in samples from four of these patients were increased fourfold or more. The fifth patient (Patient 2) was given a diagnosis and treatment within a week after the onset of symptoms, so there may not have been sufficient time for his titer to become elevated. The indirect immunofluorescent-monoclonal-antibody assay detected intracellular or extracellular amastigotes in tissue samples from all eight patients (Fig. 1). The cultures of all patients became positive (median time, 9.5 days; range, 6 to 14). Six cultures expanded sufficiently for characterization by enzyme electrophoresis; all the isolates were identified as *L. tropica*.

Treatment with sodium stibogluconate was recommended for the six patients who still had symptoms at diagnosis (20 mg per kilogram per day, infused intravenously for 30 days[16,17]). Four of these six patients completed the 30-day treatment course. The signs and symptoms of five (Patients 1, 2, 3, 5, and 7) resolved, but Patient 6 had no response to sodium stibogluconate. Severe thrombocytopenia developed in Patients 5 and 7, and the drug was discontinued after 18 and 8 days, respectively. No treatment was recommended for Patient 4, who had no symptoms; he was well after 18 months of follow-up. Patient 8, who had renal-cell carcinoma, was not treated with sodium stibogluconate. He remained symptomatic, with fatigue, malaise, nonproductive cough, and occasional low-grade fever 14 months after nephrectomy, but had no radiologic or pathological evidence of metastatic disease to account for these symptoms.

The eight patients whom we describe had a wide variety of nonspecific clinical manifestations, including prolonged fever, malaise, abdominal pain, and intermittent diarrhea. In all six patients in whom the infecting species could be differentiated, the leishmania were characterized as *L. tropica*. This organism has been reported to cause cutaneous leishmaniasis but has rarely been reported to produce systemic illness. Six of the patients were otherwise healthy and immunocompetent. No other diagnosis was confirmed in these six patients despite extensive evaluations, and five of them responded to specific therapy for leishmaniasis. Patient 7, the patient with HIV infection, had a nonspecific illness associated with HIV seroconversion. He was examined for leishmaniasis because of our high index of suspicion and an elevated titer of 1:64 on the immunofluorescent-antibody assay. In Patient 8, the localized renal-cell carcinoma was an unexpected finding during an exhaustive evaluation. Acute retroviral seroconversion and renal-cell carcinoma could explain the illnesses seen in these two patients, and we cannot conclude that their clinical presentations were due solely to leishmaniasis. Visceral leishmaniasis was included in the differential diagno-

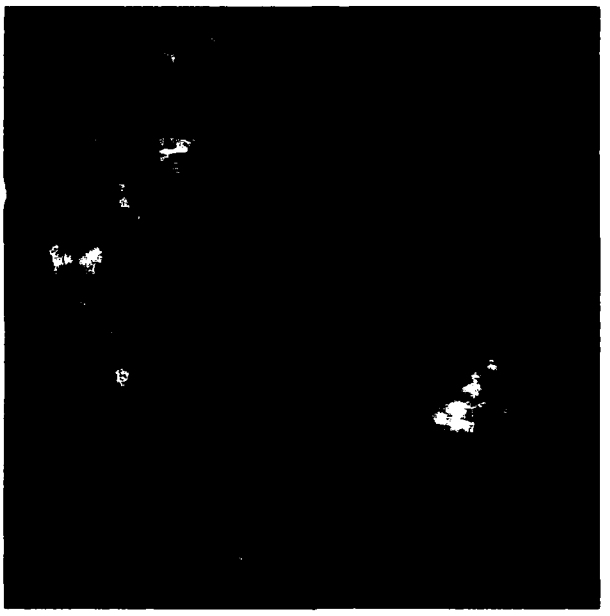

Figure 1. Intracellular Amastigotes in Mononuclear Cells from the Bone Marrow Aspirate of Patient 2, on Indirect Immunofluorescent-Monoclonal-Antibody Assay.

Amastigotes are the nonflagellated form of leishmania (3 to 5 μm in diameter) found in the mammalian host. Promastigotes are the flagellated form found in the sand fly.

sis of systemically ill soldiers because it is one of the endemic infectious diseases of Saudi Arabia that has an incubation period longer than one month[1,2] and because we were aware of the initial clinical presentation of visceral leishmaniasis, particularly in persons with no immunity to leishmania.[18,19]

The characterization of the isolates as *L. tropica* was unexpected, since this species is considered the cause of Old World cutaneous leishmaniasis.[20,21] However, isolates from two Kenyan patients with kala-azar[22] and visceral strains from Israeli and Indian patients have also been characterized as *L. tropica*.[23] A report of visceral *L. tropica* from Israel that shares certain features with *L. donovani* suggests that strains that cause visceral disease may differ from strains causing cutaneous disease.[24]

Discussion

The most severe clinical manifestation of visceral infection caused by leishmania is kala-azar, caused by *L. donovani*. However, *L. donovani* infection may also result in asymptomatic infection, acute febrile illness, or a prolonged nonspecific systemic illness that does not progress to kala-azar.[25,26] Epidemiologic studies have documented asymptomatic infections in Italy,[27,28] Kenya,[29] and Brazil.[25] The ratio of cases of infection to cases of active disease has ranged from 6.5:1, as reported by Badaró et al.,[25] to 30:1, as reported by Bryceson.[30] "Acute kala-azar" is characterized by the abrupt onset of fever, rigors, malaise, and other nonspecific symptoms as early as two weeks after infection; it may mimic malaria.[18,19]

An outbreak of visceral leishmaniasis in northern Italy in 1971 and 1972 illustrated the spectrum of infection. Some persons had asymptomatic infection, and others had a history of unexplained fever and asthenia. Many had positive leishmanin skin tests, and some had a parasitologically confirmed or histopathologically compatible diagnosis of visceral leishmaniasis.[27,28] The eight patients we describe had presentations that paralleled portions of the spectrum of *L. donovani* infection reported by the earlier observers.

Prolonged systemic illness that did not progress to kala-azar has been described in Brazil.[26] The course of acute infection due to *L. chagasi* was studied in 86 Brazilian children with leishmaniasis who had antibody seroconversion. They were divided into three clinical groups: 23 percent remained asymptomatic; 44 percent, described as having "subclinical" infection, had a prolonged illness (mean duration, 35 months) characterized by low-grade fever, malaise, weight loss, intermittent diarrhea, and hepatosplenomegaly; and the condition of the remaining 33 percent progressed to kala-azar. Some of the patients with prolonged illness had complete resolution of their symptoms, but others had a slow convalescence characterized by weakness, fatigue, lethargy, intermittent fever, adenopathy, and diarrhea. Findings at physical examination were usually limited to the reticuloendothelial system, with intermittent generalized or regional lymphadenopathy, splenomegaly, or hepatomegaly in some but not all symptomatic patients. All these patients were children. Most had major health problems predisposing them to disease, such as concurrent infections and malnutrition, and thus they are not directly comparable to our patients, who were all well-nourished adults.

The diagnosis of visceral leishmaniasis requires visualization of the intracellular, nonflagellated amastigote stage of the parasite in host tissue or of the extracellular, flagellated promastigote stage in culture.[21] However, parasitologic diagnosis can be difficult. In the study from Brazil, only 1 of 11 bone marrow aspirates from patients with "subclinical" infection and splenomegaly was positive on Giemsa staining.[26] In an Italian study, amastigotes could be demonstrated in only one of six liver-biopsy specimens.[28] Careful evaluation by experienced observers of Giemsa-stained marrow from one of our patients (Patient 2) also failed to reveal amastigotes. Amastigotes were seen in all our patients on indirect immunofluorescent-monoclonal-antibody assay, a technique with greater sensitivity than Giemsa staining of smears or culture.[8] Although very specific for the diagnosis of leishmaniasis, primary isolation of leishmanial promastigotes in culture can be difficult.[31] The sensitivity of this method varies and depends on the number of viable amastigotes in the inoculum, the parasite strain, the type of mediums used, the sample source, and the expertise of the laboratory.[32] For patients without the classic findings of kala-azar, like those we describe, who presumably have low parasite burdens, the sensitivity of primary culture may be low. There is no available diagnostic test to detect infection or early manifestations of systemic disease. For visceral leishmaniasis, the use of the classic diagnostic criterion of visualization of parasites detects only cases of disease in which the parasite burden is high.

The finding of visceral illness due to leishmania in returning troops raises at least two important clinical issues: late presentation due to prolonged incubation, and activation of latent infection in immunosuppressed persons. Incubation periods of up to 10 years have been reported for *L. donovani*.[33] Immunosuppression of any type poses an additional risk, since illness has been documented up to 20 years after exposure.[34] Leishmania are recognized as serious opportunistic pathogens in persons with HIV infection.[35-37] Leishmania may also cause disease during immunosuppression after organ transplantation[38-44] or during the administration of high doses of corticosteroids.[45] Virulent *L. major*, another Old World cutaneous parasite, persists for at least one year in the visceral organs and lymph nodes of mice after recovery from infection.[46] If *L. tropica* is also capable of surviving in a latent state, visceral leishmaniasis will need to be included in the differential diagnoses of illness in veterans of Operation Desert Storm for years to come.

Leishmanial illnesses similar to those we have described may not be recognized as such when they occur in populations in which they are endemic, because of their protean clinical manifestations, insensitive di-

agnostic tests, and infrequent examination of bone marrow for amastigotes. Another possibility is that nearly universal infection in childhood leads to resistance to disease in adult life. The exposure of more than 500,000 nonimmune adults during Operation Desert Storm may therefore have revealed more of the clinical spectrum of infection caused by *L. tropica*.

We have described a systemic illness caused by *L. tropica*. We call this illness "viscerotropic" leishmaniasis to distinguish it from "visceral" leishmaniasis, which is frequently considered to be the same as classic kala-azar.[18,47] The natural history of this illness is not yet defined, and the prevalence of infection among returning troops is not known. Diagnosis still requires an invasive procedure, such as a bone marrow aspiration or a lymph-node biopsy, and specialized laboratory support that is not widely available. This disorder should be included in the differential diagnosis of unexplained systemic illness in patients who have returned from areas of the world where leishmaniasis is endemic.

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